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     FILE 'BIOSIS, MEDLINE, CAPLUS' ENTERED AT 19:38:48 ON 08 JUN 2005
L1          7184 S GPCR
L2           388 S L1 AND LIBRARY
L3            25 S L2 AND MUTATION
L4             8 S L3 AND SCREEN
L5             4 DUP REM L4 (4 DUPLICATES REMOVED)
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L5 ANSWER 1 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 1
TI Random mutagenesis of the M3 muscarinic acetylcholine receptor expressed in yeast - Identification of second-site **mutations** that restore function to a coupling-deficient mutant M3 receptor.
PY 2005
SO Journal of Biological Chemistry, (February 18 2005) Vol. 280, No. 7, pp. 5664-5675. print.
CODEN: JBCHA3. ISSN: 0021-9258.
TI Random mutagenesis of the M3 muscarinic acetylcholine receptor expressed in yeast - Identification of second-site **mutations** that restore function to a coupling-deficient mutant M3 receptor.
AB The M, muscarinic receptor is a prototypical member of the class A family of G protein-coupled receptors (**GPCRs**). To gain insight into the structural mechanisms governing agonist-mediated M, receptor activation, we recently developed a genetically modified yeast strain (Saccharomyces cerevisiae) which allows the efficient screening of large **libraries** of mutant M3 receptors to identify mutant receptors with altered/novel functional properties. Class A **GPCRs** contain a highly conserved Asp residue located in transmembrane domain II (TM II; corresponding to Asp-113 in the rat M3 muscarinic receptor) which is of fundamental importance for receptor activation. As observed previously with other **GPCRs** analyzed in mammalian expression systems, the D113N point **mutation** abolished agonist-induced receptor/protein coupling in yeast. We then subjected the D113N mutant M, receptor to PCR-based random mutagenesis followed by a yeast genetic **screen** to recover point **mutations** that can restore G protein coupling to the D113N mutant receptor. A large scale screening effort led to the identification of three such second-site suppressor **mutations**, R165W, R165M, and Y250D. When expressed in the wild-type receptor background, these three point **mutations** did not lead to an increase in basal activity and reduced the efficiency of receptor/G protein coupling. Similar results were. . . are located at the cytoplasmic ends of TM III and TM V, respectively, are also highly conserved among class A **GPCRs**. Our data suggest a conformational link between the highly conserved Asp-113, Arg-165, and Tyr-250 residues which is critical for receptor. . .
IT Major Concepts
Biochemistry and Molecular Biophysics; Methods and Techniques
IT Chemicals & Biochemicals
G-protein-coupled receptors [**GPCRs**]: Class A family, transmembrane domain II, transmembrane domain III, transmembrane domain V; M-3 muscarinic receptor
IT . . . & Equipment
PCR [polymerase chain reaction]: genetic techniques, laboratory techniques; random mutagenesis: genetic techniques, laboratory techniques
IT Miscellaneous Descriptors
point **mutation**

L5 ANSWER 2 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
TI A high throughput cell-based **screen** for identification of putative Alzheimer's disease modifying drugable genes that modulate amyloid levels.
PY 2003
SO Society for Neuroscience Abstract Viewer and Itinerary Planner, (2003) Vol. 2003, pp. Abstract No. 445.11. http://sfn.scholarone.com. e-file. Meeting Info.: 33rd Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 08-12, 2003. Society of Neuroscience.
TI A high throughput cell-based **screen** for identification of putative Alzheimer's disease modifying drugable genes that modulate amyloid levels.
AB Genetic linkage studies revealed segregation of **mutations** in APP and in APP-processing genes PS1 and PS2 with Alzheimers disease pathology and clinical phenotype. These findings underscored the. . . Our state of the art arrayed adenoviral platform allows automated, highly efficient induction of single genes into mammalian cells. Pre-selected

libraries of adenoviruses holding cDNAs or siRNA sequences of drugable genes are applied that knock in or knock down genes, respectively.. . . secreted Abeta levels reproducibly, both in the knock-in and knock-down approach. Genes of different drugable classes are screened, such as **GPCRs**, NHR, kinases and others. Up to now, 3 new **GPCRs** are identified that upon overexpression modulate Abeta levels in conditioned medium in a cell specific manner. In conclusion, combining these. . .

IT . . .
and mental disorders, nervous system disease
Alzheimer Disease (MeSH)

IT Diseases
infection: infectious disease
Infection (MeSH)

IT Chemicals & Biochemicals
A-beta1-42; BACE; **GPCR**; NHR; PS1; PS2; amyloid; genes; siRNA

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TI MECHANISMS OF DELTA OPIOID RECEPTOR ACTIVATION.

PY 2002

SO Society for Neuroscience Abstract Viewer and Itinerary Planner, (2002) Vol. 2002, pp. Abstract No. 515.7. http://sfn.scholarone.com. cd-rom. Meeting Info.: 32nd Annual Meeting of the Society for Neuroscience. Orlando, Florida, USA. November 02-07, 2002. Society for Neuroscience.

AB. . . (CAM) receptors. We have optimized PCR conditions to randomly mutate the entire hDOR cDNA under conditions that statistically introduce one point-**mutation** per receptor molecule. We have transiently expressed the receptor **library** into HEK 293 cells and used a high-throughput reporter gene assay in conjunction with the inverse agonist ICI174864 to identify. . . receptors. Out of a screening of 3000 clones, we obtained several mutant receptors and identified the nature and localization of **mutations** by DNA sequencing. Mutants were also transfected into COS cells to confirm constitutive activity using another functional assay (GTPgammaS). Interestingly, **mutations** are organized in discreet microdomains and allow to speculate on possible mechanisms for hDOR activation using 3D-modelling. This strategy offers. . . draw a general picture of receptor activation. Both the approach and some of the conclusions may be applicable to other **GPCRs**. Mutant receptors will be useful to **screen** for compounds with inverse agonist properties.

IT Major Concepts
Molecular Genetics (Biochemistry and Molecular Biophysics); Neurology (Human Medicine, Medical Sciences)

IT Chemicals & Biochemicals
DNA; **GPCR**; ICI174864; constitutively active mutant receptor: expression; delta opioid receptor: activation; h-delta opioid receptor cDNA: activation

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DUPLICATE 2

TI A limited spectrum of **mutations** causes constitutive activation of the yeast alpha-factor receptor.

PY 2000

SO Biochemistry, (June 13, 2000) Vol. 39, No. 23, pp. 6898-6909. print. CODEN: BICHAW. ISSN: 0006-2960.

TI A limited spectrum of **mutations** causes constitutive activation of the yeast alpha-factor receptor.

AB Activation of G protein coupled receptors (**GPCRs**) by binding of ligand is the initial event in diverse cellular signaling pathways. To examine the frequency and diversity of **mutations** that cause constitutive activation of one particular **GPCR**, the yeast alpha-factor receptor, we screened **libraries** of random **mutations** for constitutive alleles. In initial **screens** for mutant receptor alleles that exhibit signaling in the absence of added ligand, 14 different point **mutations** were isolated. All of these 14 mutants could be further activated by alpha-factor. Ten of the mutants also acquired the. . . of endogenous alpha-factor present in MATa cells. The strongest constitutively active receptor alleles were

recovered multiple times from the mutational **libraries**, and extensive mutagenesis of certain regions of the alpha-factor receptor did not lead to recovery of any additional constitutive alleles. Thus, only a limited number of **mutations** is capable of causing constitutive activation of this receptor. Constitutive and hypersensitive signaling by the mutant receptors is partially suppressed. . .